

TURFGRASS

Carbon Isotope Discrimination of Three Kentucky Bluegrass Cultivars with Contrasting Salinity Tolerance

Y. L. Qian,* R. F. Follett, S. Wilhelm, A. J. Koski, and M. A. Shahba

ABSTRACT

We evaluated leaf C isotope discrimination as affected by salinity among three Kentucky bluegrass (*Poa pratensis* L.) cultivars that differ in their salt tolerance. 'Moonlight', 'NorthStar', and 'P-105' Kentucky bluegrass (KBG) were grown in solution culture and exposed to salinity levels of 2.0, 5.0, 8.0, 11.0, and 14.0 dS m⁻¹ for 12 wk. All cultivars exhibited increased leaf firing with increasing salinity. However, Moonlight and NorthStar exhibited less leaf firing than P105 at all salinity levels. The salinity levels that caused 25% shoot growth reduction were 4.9 dS m⁻¹ for NorthStar and Moonlight and 4.1 dS m⁻¹ for P105, indicating that Moonlight and NorthStar have better salinity tolerance than P105. When salinity level was in the range of 2.0 to 8.0 dS m⁻¹, Moonlight produced 1.9-fold more root mass than NorthStar, and NorthStar exhibited 3.9-fold more root mass than P105. When salinity exceeded 8.0 dS m⁻¹, NorthStar had similar root mass as Moonlight, and both showed greater root mass than P105. Cultivar P105 had a higher C isotope discrimination (Δ) than Moonlight and NorthStar under nonsaline conditions (<2 dS m⁻¹) but a lower Δ than Moonlight and NorthStar at 11.0 dS m⁻¹ salinity. The great reduction in Δ of P105 as salinity increased suggests that salinity induced a greater degree of stomatal resistance that provided less opportunity for discrimination against the heavier isotope. Carbon isotope discrimination may serve as a useful selection criterion in breeding efforts to develop salt tolerant KBG.

SALT PROBLEMS in turfgrass sites are becoming more common for a number of reasons: accelerated urban development in arid and semiarid western regions where salinity problems are common, the use of reclaimed wastewater or other irrigation waters containing salts, seawater intrusion into turf facilities located on coastal sites, water conservation, and road deicing (Carrow and Duncan, 1998). Limited water resources in the western states increasingly force turf facilities to use low quality water for irrigation. Water conservation efforts can further increase soil salinity levels due to reduced leaching. Selection and use of relatively salt tolerant cultivars will benefit sites where soil and water salinity are marginally high.

Although KBG (one of the most widely used cool-season turfgrasses in the United States) is generally considered to be salt-sensitive, studies have found that cultivars of KBG differ in their salinity tolerance, with

tolerant cultivars showing less growth reduction than sensitive cultivars under saline conditions (Qian et al., 2001; Suplick-Ploense et al., 2002). Growth reduction under salt stress could be attributed to excessive ion accumulation in the plant tissue and/or to water stress due to low external osmotic potential resulting from salinity.

Selection for salt tolerant species and/or cultivars in the field is time consuming and difficult due to spatial and temporal variations of salinity level and salt composition. Measurement of C isotopic composition and Δ in nonstressed, controlled environments has been found to be related to water use efficiency in the field, production under water stress conditions, and plant-environmental interactions in many cool-season grasses (Farquhar and Richards, 1984; Johnson et al., 1990; Johnson and Bassett, 1991). Ebdon et al. (1998) reported that C isotope discrimination is a useful criterion for assessing water use efficiency in KBG. It is unknown if Δ is associated with KBG salt tolerance and if low Δ of KBG grown under nonsaline conditions would provide an indirect indication of salt tolerance. If Δ can be shown to reliably predict salinity tolerance in KBG, it would provide a useful tool for rapid screening of KBG germplasm for salinity tolerance.

Approximately 1.1% of the C in the biosphere is in the form of the stable isotope ¹³C. The ratio of ¹³C/¹²C in plant dry matter of C₃ plants is lower than the ratio of the source air due to plants' discriminations against ¹³C during diffusion of CO₂ through stomata and during the process of CO₂ fixation by ribulose-1,5-bisphosphate carboxylase (rubisco). The degree of discrimination against ¹³C differs among species and varies with environments. Higher degree of discrimination results in a greater degree of depletion in ¹³C in plant tissue in relation to CO₂ in the air.

Two nomenclatures have been used to describe the ¹³C content of plant materials. Isotope composition ($\delta^{13}\text{C}$) is a value indicating whether the sample has a higher or lower ¹³C/¹²C isotope ratio than PDB, a limestone standard from Pee Dee, SC (Farquhar et al., 1988). Therefore, isotope composition of a plant tissue (δp) can be expressed as:

$$\delta\text{p} (\%) = \left[\frac{(^{13}\text{C}/^{12}\text{C})_{\text{plant}}}{(^{13}\text{C}/^{12}\text{C})_{\text{standard}}} \right] - 1 \times 1000 \quad [1]$$

Carbon isotope discrimination is a measure of the C

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Abbreviations: C_i/C_a, the ratio of intercellular and atmospheric CO₂ concentration; EC, electrical conductivity; KBG, Kentucky bluegrass; Δ , carbon isotopic discrimination.

isotopic composition in plant material relative to the value of the same ratio in the air on which plants feed:

$$\Delta = (\delta a - \delta p)/(1 + \delta p) \quad [2]$$

where Δ represents carbon isotope discrimination, δa represents C isotope composition in the source air, and δp represents C isotope composition in the plant tissue. A wealth of work has been published showing strong negative relationship between Δ and water use efficiency in plants (Condon et al., 1987; Ehdaie et al., 1991; Johnson and Bassett, 1991; Johnson et al., 1990).

Theory published by Farquhar et al. (1989) and Farquhar and Richards (1984) indicates that C discrimination in leaves of cool-season plants can be expressed in relationship to CO_2 concentrations inside and outside the leaf in its simplest form as:

$$\Delta = a + (b - a)C_i/C_a \approx 4.4 + (27 - 4.4)C_i/C_a \quad [3]$$

where a is discrimination that occurs during diffusion of CO_2 through the stomata (4.4‰), b is discrimination by rubisco (27‰), and C_i/C_a is the ratio of the leaf intercellular CO_2 concentration to that in the atmosphere. Equation [3] shows a direct and linear relationship between Δ and C_i/C_a . Therefore, measurement of Δ gives an estimation of the assimilation-rate-weighted value of C_i/C_a . A lower C_i/C_a ratio may result either from higher rates of photosynthetic activity or from stomatal closure induced by water stress.

Objectives of the present study were to (i) compare plant growth and salinity tolerance of three KBG cultivars and (ii) examine variations of Δ values among three KBG cultivars over a range of salinity levels.

MATERIALS AND METHODS

Plant Culture

The experiment was conducted in the greenhouse from December 2001 to May 2002 using a solution culture system. Plants were grown under natural light in the greenhouse with photosynthetically active radiation from $150 \mu\text{mol m}^{-2} \text{s}^{-1}$ on cloudy days to $1150 \mu\text{mol m}^{-2} \text{s}^{-1}$ on sunny days. General procedures were described in previous publications (Qian et al., 2000, 2001; Suplick-Ploense et al., 2002). Briefly, sward pieces of Moonlight, NorthStar, and P105 measuring 10 cm in diam. were sampled from 3-yr-old field plots. Sod pieces were hand-washed to remove soil and then planted in a hydroponic system with complete nutrient solution (Peters Excel, Cal-Mag, Scotts-Sierra Hortic. Products Co., Marysville, OH).

Salinity treatments were applied by adding instant ocean salt (Aquarium Syst., Mentor, OH) gradually during a 3-d period to obtain electrical conductivity (EC) values of 5.0, 8.0, 11.0, and 14.0 dS/m, measured by an EC meter (Model CO150, Hach Co., Loveland, CO). Nutrient solution (EC = 2.0 dS m^{-1}) without the addition of ocean salts served as the control. Grasses were exposed to final salinity treatments for a period of 12 wk. During this period, solution EC of all treatment tanks was measured every 2 to 3 d and adjusted when necessary.

Data Collection

Leaf firing percentage was determined weekly, beginning at 5 wk after initiation of salinity treatments, by visually esti-

imating total percentage of chlorotic leaf area. Means of leaf firing over time are presented for each salinity level. Visual turf quality was rated by the end of the experiment on a 1 to 9 scale, where 1 = brown, thin, dead turf; 9 = dense, uniform, green, nonstressed turf; and 6 = minimal acceptable quality. Throughout the experiment, grasses were clipped weekly to a height of 2.5 cm. Clipping yields grown during the week were harvested at 2, 4, and 10 wk after the initiation of salinity treatments and dried at 70°C for 48 h for dry weight determination. Following the final clipping event at Week 12, the grass sward was harvested and divided into verdure and roots. Each fraction was then dried in a force-draft oven at 70°C for 48 h to determine dry mass.

Carbon Isotope Analysis

At 2, 4, 6, and 8 wk after salinity treatment, leaf blades grown during the previous week were harvested, dried at 70°C for 48 h, and then finely ground (<100 μm). The $^{13}\text{C}/^{12}\text{C}$ ratio of the leaf dry matter was determined by isotope ratio mass spectrometer (IRMS) in the ARS Soil-Plant-Nutrient Research Unit in Fort Collins. Carbon isotope discrimination was calculated as described in Eq. [2] assuming a $^{13}\text{C}/^{12}\text{C}$ ratio of CO_2 in the air in the greenhouse equal to 8.4‰ on the PDB scale (Mook et al., 1983).

Data Analysis

Experimental design was a split plot with four replications, with salt treatment (tank) as the main plot effect and cultivars (pots) within each tank as subplot effects. Salinity and cultivar effects were determined by analysis of variance (SAS Inst., 1989). Treatment means were separated using Fisher's protected LSD. Regression analysis was performed to define linear or quadratic relationships between each variable and the salinity level.

RESULTS

Turf Quality

The analysis of variance showed significant effects of salinity, cultivar, and their interaction in affecting leaf firing (Table 1). With increasing salinity, all cultivars exhibited increased leaf firing though leaf firing increased more rapidly in P105 than Moonlight and NorthStar (Table 2). At all salinity treatments, P105 exhibited higher levels of leaf firing than both NorthStar and Moonlight.

Salinity reduced turf quality in all cultivars linearly. However, the reduction slope was higher in P105 than Moonlight and NorthStar. Regression analysis predicted that turf quality would fall to below the acceptable level (i.e., a quality rating <6) when salinity level is higher than 5.9, 6.0, and 3.0 dS m^{-1} for Moonlight, NorthStar, and P105, respectively.

Growth Responses

Clipping yields were influenced by salinity, cultivar, and their interaction (Table 1). Mean weekly clipping yield decreased linearly with increasing salinity for all cultivars (Table 2). Under nonsaline conditions, cultivars did not differ in clipping yield, whereas at 8 and 14 dS m^{-1} , Moonlight and NorthStar produced 55 and 61% higher clipping yields, respectively, than P105. Regression predicted that 25% shoot growth reduction

Table 1. Analyses of variance with mean squares and treatment significance levels.

Variable	Salt	Block	Salt × block	Cultivar	Salt × cultivar
Leaf firing (%)	6241†	86 ns‡	100*	4271†	334***
Turf quality (1–9 scale)	35.6†	0.1 ns	0.1 ns	17.4†	0.5**
Clipping yield (g)	0.047†	0.0002 ns	0.0004 ns	0.0040***	0.0008*
Root mass (g)	0.16†	0.001 ns	0.006 ns	0.56†	0.07†
Verdure (g)	0.56*	0.15*	0.07 ns	1.18†	0.10*
Isotope discrimination (Δ)	17.03†	0.5 ns	0.44 ns	2.42***	0.89**

* Significant at the 0.05 level.

** Significant at the 0.01 level.

*** Significant at the 0.001 level.

† Significant at the 0.0001 level.

‡ ns, not significant.

occurred at 4.9 dS m⁻¹ for NorthStar and Moonlight and at 4.1 dS m⁻¹ for P105.

Except at 14 dS m⁻¹, Moonlight exhibited greater verdure than NorthStar. P105 produced less verdure than Moonlight across all salinity levels except for 5 dS m⁻¹. At 14 dS m⁻¹, both Moonlight and NorthStar had greater verdure than P105.

Root growth of three cultivars responded differently to increasing salinity, being linear in NorthStar but curvilinear in Moonlight and P105 (Table 2). As salinity level increased from control (2.0 dS m⁻¹) to 5.0 dS m⁻¹, root mass of NorthStar remained the same, and Moonlight exhibited a trend of increasing root mass. With further increasing salinity, root growth decreased. In contrast, root growth of P105 decreased greatly as salinity increased to 5.0 dS m⁻¹. At control and 5.0 and 8.0 dS m⁻¹ salinity levels, Moonlight produced 1.9- and 13-fold greater root mass than NorthStar and P105, respectively. NorthStar produced 3.9-fold greater root mass than P105 when salinity ranged from control to 8.0 dS m⁻¹. At 11.0 and 14.0 dS m⁻¹, Moonlight and NorthStar pro-

duced 10 and 50 times greater root mass than P105, respectively.

Carbon Isotope Discrimination

As reported for other C₃ species (Guy et al., 1986; Brugnoli and Lauteri, 1991; Gibberd et al., 2003), KBG grown under salinity in this study was enriched in the heavier isotope C compared with their control plants (Fig. 1). The decrease in Δ reflected the decrease in C_v/C_a resulting from greater stomatal closure caused by high salinity. However, our results indicated that the effect of salinity on Δ was greater in P105 than in Moonlight and NorthStar, as demonstrated by the steeper reduction slope in P105 than in Moonlight and NorthStar (Fig. 1).

Under nonsaline conditions, NorthStar and Moonlight exhibited lower Δ than P105, suggesting Moonlight and NorthStar had higher water use efficiencies and were more conservative in utilizing water under nonsaline and nonstressed conditions (Farquhar et al., 1988).

Table 2. Effect of salinity on leaf firing, turf quality, clipping yield, verdure, and root mass of Moonlight, NorthStar, and P105 Kentucky bluegrass.

Cultivar	Salinity (dS m ⁻¹)					Linear or quadratic relationship
	2	5	8	11	14	
Leaf firing						
%						
Moonlight	0.1a†	1.5a	5.4a	29.5a	37.5a	Y = 2.19x
NorthStar	0.0a	2.9a	12.5a	28.4a	30.0a	Y = 2.03x
P105	1.2b	18.7b	38.7b	53.7b	53.7b	Y = 4.16x
Turf quality						
1–9 scale, where 9 = best						
Moonlight	7.8a	6.9a	6.1a	4.9a	4.6a	Y = 8.3 – 0.39x
NorthStar	7.9a	7.2a	5.9a	5.0a	5.1a	Y = 8.0 – 0.32x
P105	7.1b	5.7b	4.6b	3.7b	3.7b	Y = 7.4 – 0.48x
Clipping yield						
g per cup						
Moonlight	0.187	0.136ab	0.123a	0.071	0.55a	Y = 0.195 – 0.010x
NorthStar	0.169	0.156a	0.104a	0.067	0.061a	Y = 0.181 – 0.009x
P105	0.178	0.111b	0.073b	0.062	0.036b	Y = 0.174 – 0.0105x
Verdure						
g per cup						
Moonlight	1.65a	1.42a	1.40a	1.24a	1.06a	Y = 1.72 – 0.045x
NorthStar	1.21b	1.10b	1.07b	0.92b	1.04a	Y = 1.19 – 0.017x
P105	1.24b	1.22ab	0.80c	0.96b	0.65b	Y = 1.39 – 0.050x
Root mass						
g per cup						
Moonlight	0.413a	0.495a	0.495a	0.066a	0.055a	Y = 0.40 + 0.032x – 0.0044x ²
NorthStar	0.176b	0.173b	0.121b	0.053a	0.049a	Y = 0.21 – 0.012x
P105	0.078c	0.013c	0.005c	0.005b	0.001b	Y = 0.02 + 0.006x – 0.00037x ²

† Means followed by same letter within the same parameter and column are not significantly different at the 0.05 probability level using Fisher's LSD test.

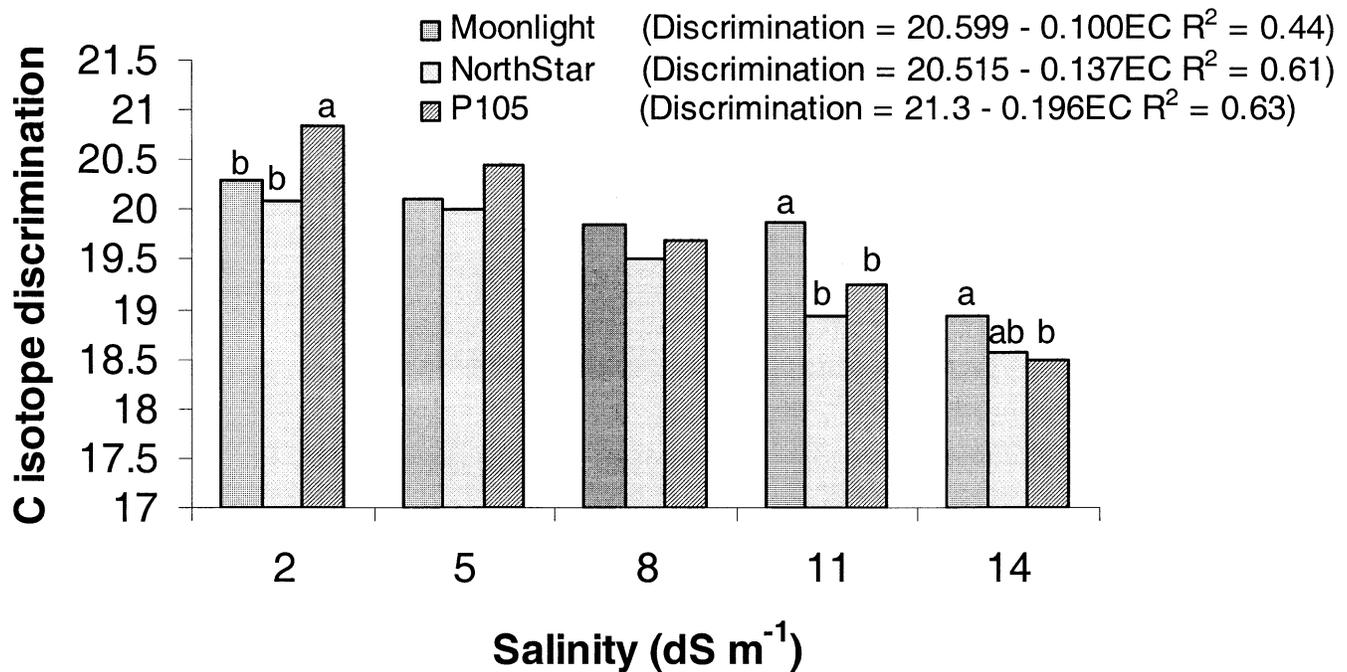


Fig. 1. Effect of salinity between 2.0 and 14.0 $dS\ m^{-1}$ on C isotope discrimination (Δ) of Moonlight, NorthStar, and P105 Kentucky bluegrass. Columns labeled with different letters are significantly different, within a given salinity level, at 0.05 probability using Fisher's LSD test. EC, electrical conductivity.

However, under high-salinity treatments (11 and 14 $dS\ m^{-1}$), Moonlight and NorthStar had a higher Δ than P105. This was the result of greater access to moisture at high salinity due to extensive rooting, reflecting the ability of Moonlight and NorthStar to maintain greater stomatal conductance than P105 at high salinity. In response to the salinity increase from control to 14 $dS\ m^{-1}$, Δ of P105 decreased by 2.4 ‰ whereas the reduction for Moonlight and NorthStar were only 1.4 to 1.5‰. Brugnoli and Lauteri (1991) observed a 2.7‰ decrease in Δ for kidney bean (*Phaseolus vulgaris* L.) as NaCl concentration in the growth medium increased from 0 to 50 mM. Gibberd et al. (2002, 2001) observed Δ reductions of 1.4‰ in carrot (*Daucus carota* L.) and 4.7‰ in grapevine (*Vitis vinifera* L.) as salinity (NaCl) increased from control (0 mM) to 80 mM. Using Eq. [3], we can estimate the average C_i/C_a among different salinity treatments. The reduction in Δ as salinity increased from control to 14 $dS\ m^{-1}$ corresponded to a decrease in C_i/C_a from 0.73 to 0.62 in P105 and from 0.70 to 0.64 in Moonlight and NorthStar. A lower C_i/C_a ratio could result either from stomatal closure induced by water stress or from higher rates of photosynthetic capacity or a combination of both (Condon et al., 2002). However, considering the higher leaf firing and lower shoot growth, it was unlikely that a higher photosynthetic activity occurred in P105 compared with Moonlight and NorthStar. Under salinity stress, the lower C_i/C_a in P105 reflected a greater degree of stomatal limitation of photosynthetic capacity than cultivars Moonlight and NorthStar. Stomatal closure at least in part contributed to the declines in growth and turf quality and increases in leaf firing. Therefore, a great reduction in Δ as salinity increases indicates salinity intoler-

ance and, in particular, salinity-induced osmotic stress intolerance.

Based on turf quality, shoot and root growth, and the degree of reduction in Δ , we found that Moonlight and NorthStar are more salt tolerant than P105, which is in agreement with the findings of Rose-Fricker and Wipff (2001). Concurrently, we found that Moonlight and NorthStar exhibited lower Δ than P105 under nonsaline conditions. This suggested that a lower Δ (an indicator of higher water use efficiency) under nonsaline conditions may provide an indirect indication of KBG salt tolerance. Though not tested in the current study, in a previous salt tolerance study involving KBG, Qian et al. (2001) reported that 'Kenblue' KBG was very salt sensitive. Ebdon et al. (1998) found that Kenblue KBG had higher Δ than eight other KBG cultivars tested under nonstressed conditions. It appears that salt-sensitive cultivars may exhibit high discrimination under nonsaline conditions. However, this relationship needs to be confirmed by testing more cultivars.

In summary, our results demonstrated that Moonlight and NorthStar are more salt tolerant than P105, evidenced as they exhibit less leaf firing, less reductions in shoot and root growth, and Δ under high-salinity conditions. Our results suggest that there are two ways in which Δ maybe useful in assessing KBG salinity tolerance, the degree of reduction in Δ as salinity increase and the Δ baseline under nonsaline conditions. Considering the high precision and repeatability for Δ measurements (Ebdon et al., 1998), C isotope technique is promising to serve as a selection criterion in breeding efforts to develop salt-tolerant KBG.

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